
FULL CONTENTS

[Claim(s)]
[Claim 1] Glycoprotein 39 gene.
[Claim 2] The gene containing the base sequence which carries out the code of the amino acid sequence shown by the base sequence which carries out the code of the amino acid sequence shown by the arrangement number 1, and the arrangement number 2 according to claim 1.
[Claim 3] The gene containing the base sequence for 3' end flank shown by the base sequence and the arrangement number 2 for 5' end flank shown by the arrangement number 1 according to claim 1.

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the gene containing the base sequence which carries out the code of the core protein of the mucin glycoprotein 39 of Homo sapiens origin useful as a tumor marker, an immunopathy marker, or various inflammatory disease markers still in detail about mucin glycoprotein 39 new gene.
[0002]
[Description of the Prior Art] It is known that different glycoconjugate from a normal cell, such as glycoprotein and glycolipid, exists in the cell membrane of the cell which generally cancerated. Moreover, it faces diagnosing Gand and the method of measuring the protein antigen specifically produced in a cancer patient and a carbohydrate antigen is performed. As the example, the carcinoembryonic antigen (CEA) and alpha fetoprotein, [Muramatsu **, Japan clinical [by which diagnosis of the cancer of the digestive system by measurement of CA19-9 etc. is known], 44, and p.337-344 (1986); Reiji Kanaki, clinical pathology, 35, and p.1247-1264 (1986); Medical Ayumi, 106 volumes, No. 5, the 5th Saturday special edition, 235-250page(1978)].
[0003]
[Problem to be solved by the invention] However, the kind of applicable cancer is restricted comparatively, or the diagnosis of Gand using the various conventional cancer antigen measurement has problems, like a cross reaction with other diseases, such as a healthy person and hepatitis, starts, and the diagnosis applicable to the cancer of a more extensive kind or a diagnosis with high specificity is desired. Moreover, an accurate diagnosis is desired also in the various illness and the various various

inflammatory diseases based on an immunopathy response. And it is anxious for development of the gene which carries out the code of the new glycoprotein which can serve as the tumor marker, the immunopathy marker, or the various inflammatory disease markers which can be used for diagnosis of this disease, and its core protein.
[0004] On the other hand, the sugar chain of mucin and the structure analysis of core protein which have been discovered in various cancer organizations etc. progress in recent years. Relevance with various diseases including cancer has attracted attention [Bhavanandan, V.P., Glycobiology, 1, and 493-503 (1991)].
[0005]
[Means for solving problem] Then, the place which has inquired paying attention to the glycoprotein discovered to Homo sapiens gastric cancer cell surface in order for this invention persons to solve the above-mentioned technical problem, The gene which carries out the code of the core protein of the new mucin glycoprotein 39 with which the application to diagnosis of a tumor, immunopathy, or various inflammatory diseases is expected is found out. PORIMORU Fick by whom this was found out in a breast cancer or a pancreatic cancer EPISE -- real Although logy [a high gay] was indicated to be mucin (PEM), it showed clearly that it is clearly different new mucin, and this invention was completed.
[0006] That is, this invention offers glycoprotein 39 gene which carries out the code of the core protein of the new mucin of Homo sapiens origin.
[0007] this invention gene contains the base sequence which carries out the code of the amino acid sequence shown by the base sequence which carries out the code of the amino acid sequence shown, for example by the arrangement number 1, and the arrangement number 2, base sequences complementary to these amino acid sequences, or those both. In addition, in an arrangement table, the lower berth of a base sequence is an amino acid sequence presumed from the base sequence of the upper row.
[0008] Glycoprotein 39 gene of this invention is prepared as follows, for example. That is, they are all the RNA from the cell which has discovered glycoprotein 39 first. It dissociates, and after refining mRNA and compounding cDNA with a conventional method from this, the library which included this in the expression vector is built. Subsequently, the clone which has glycoprotein 39 gene from this cDNA library using anti-glycoprotein 39 antibody is chosen, and glycoprotein 39 gene of this invention is obtained. Next, it explains in detail about the process of the above-mentioned this invention gene.
[0009] [1] All the construction RNA of a cDNA library As a tissue cell used for extraction, a Homo sapiens gastric cancer organization or the cell strain already established as cellular in, For example, gastric cancer cell strain KATO-III [Sekiguchi M. and Sakakibara K.and Fujii G..(1978) Jpn.J.Exp. Med., 48, and p.61-68] is mentioned.
[0010] RNA Extraction Guanidine isothiocyanate mixed liquor or a suitable surface active agent, [with for example, physical methods, such as a method, freeze thawing, etc. which use a homogenizer using SDS, NP-40, triton X-100, deoxycholic acid, etc.,] Chromosomal DNA after destroying and solubilizing a cell partially or completely Using a mixer or glass syringes, such as Pori thoron, it shears to some extent and is performed after that by operation of classifying protein and nucleic acid fractionation. Generally the CsCl superposition method [Chirgwin, J.M., et al., Biochemistry, 18, and p.5294 (1979)] which uses phenol chloroform extraction or ultracentrifuge is used especially for this operation.
[0011] Moreover, in the describing [above] all directions method, it is RNase. RNA to depend It is RNase in order to prevent decomposition. It is good to add inhibitor, for example, heparin, a polyvinyl sulphate, a diethylpyrocarbonate, a PANAJIUMU complex, bentonite, maca LLoyd's, etc.

[0012] RNA obtained according to the above-mentioned extraction operation from -- [separation of mRNA, and refining] the method of using adsorption columns, such as for example, oligo dT-cellulose (Colaborative Research Inc.) and Pori U-sepharose (Pharmacia Corp.), for an extract -- or it can carry out with a batch method.

[0013] The refining mRNA obtained by the above is usually unstable and stable complementary DNA (cDNA). It is replaced with a mold, and in order to enable amplification of an objective gene, it connects with the vector of microorganism origin. Generally conversion to in vitro cDNA of Above mRNA, i.e., synthesis of cDNA, can be performed as follows.

[0014] Namely, Oligo dT is first made into primer (any of the oligo dT of isolation or the oligo dT already added to vector primer are sufficient as this primer). The complementary single strand cDNA is compounded to mRNA under existence of dNTP (dATP, dGTP, dCTP, or dTTP) using reverse transcriptase by using mRNA as a mold. The following steps differ as the following respectively by whether the oligo dT of isolation was used in the above, or the oligo dT added to vector primer was used.

[0015] In the case of the former, alkali treatment etc. decomposes, mRNA used as the mold is removed, and it is a single stranded DNA after that. It is considered as a mold and they are reverse transcriptase or DNA. Polymerase is used and it is double stranded DNA. It creates. Next, double stranded DNA obtained Both ends are processed by exonuclease and it is the suitable linker DNA for the each. Or two or more bases of the combination in which annealing is possible are added, and this is included in a suitable vector. According to the vector to be used, this A well-known method [Young, R.A.et al., in" DNA Cloning, Vol.1", and p.49 (1985)], for example, young people's method, or method [Gubler, U.and Hoffman, B.J.Gene, 25, and p.263 (1983)] of GUBURA and Hoffmann etc. -- it is carried out by using it. Moreover, if a commercial cDNA synthesis kit is used for synthesis of Above cDNA, it can carry out easily.

[0016] Especially restriction chooses a phage vector, a plasmid vector, etc. of a lambdagt system suitably according to a host, or the vector can combine and use it, although not carried out. lambdagt10 and lambdagt11 grade can be illustrated as a vector used here, and the method of using lambdagt10 and lambdagt11 as a vector can be performed according to said young people's method. .

[0017] By making the cDNA recombinant included in the phage vector of a lambdagt system react with in-vitro packaging liquid, it becomes cDNA recombinant phage and the cDNA library of lambdagt10 or lambdagt11 is built. Creation of the above-mentioned lambdagt system phage library can be easily performed, if lambdagt10 of marketing or a lambdagt11cDNA cloning kit is used.

[0018] Moreover, the open circular plasmid which added the same linker as the above while mRNA had been made to remain as a mold in the case of the latter, Linker DNA (DNA including the field and the transcriptional promoter field of mRNA which can often carry out independence reproduction in an animal cell a fragment can use) under dTNP existence after carrying out annealing and considering it as the shape of a ring closure RNase DNA Polymerase is made to live together and it is DNA about mRNA. It replaces by a chain and is perfect plasmid DNA. It can create.

[0019] Like the above, the cDNA recombinant plasmid which are obtained by carrying out is introduced into a host microorganism, and the transformation of this microorganism is carried out. Although Escherichia coli (Escherichia coli) is typical as a host microorganism, it is not limited to in particular this, in addition a Bacillus subtilis (Bacillus subtilis), yeast (Saccharomyces cervisiae), etc. can be used.

[0020] DNA As the method of the transformation by the introduction and this to a host microorganism,

the methods generally used, for example, the cell which is mainly in a logarithmic growth phase, are collected, and it is CaCl2. It processes and is DNA automatically. It changes into the status of being easy to incorporate, and the method into which a plasmid is made to incorporate can be adopted. It is MgCl2 in order to raise the efficiency of a transformation further in the above-mentioned method as usually known. RbCl can also be made to live together further. Moreover, spheroplast or the method of carrying out a transformation, after protoplast-izing can also adopt a microbial cell.
[0021] [2] As a method of electing the stock which contains cDNA which carries out the code of the core protein of this invention glycoprotein 39 from the transformant obtained by the selection above of glycoprotein 39 gene clone, the various methods shown below, for example are employable.
[0022] (1) Eye ****** elect using the antibody to the core protein of lectin joint glycoprotein containing this invention glycoprotein 39, cDNA is included in the vector which may discover protein within the transformant. Protein is made to produce within the transformant, the polypeptide production stock of lectin joint glycoprotein containing this invention glycoprotein 39 is detected using the second antibody to the antibody and this antibody to core protein of lectin joint glycoprotein containing this invention glycoprotein 39, and the purpose stock is obtained.
[0023] (2) Cultivate the method transformant which is made to produce polypeptide of this invention glycoprotein 39, and is screened by an animal cell. A gene is proliferated and an animal cell is transfected in that gene (in this case). Self-renewal may be possible and any of a plasmid which is integrated for a plasmid or animal cell chromosome including a mRNA transcriptional promoter field are sufficient. By making the protein by which the code was carried out to the gene produce, and detecting polypeptide of the lectin joint glycoprotein which contains this invention glycoprotein 39 using the antibody to the core protein of lectin joint glycoprotein containing this invention glycoprotein 39 The stock which has cDNA which carries out the code of the polypeptide portion of target this invention glycoprotein 39 from the original transformant is elected.
[0024] (3) [cDNA obtained from the method transformant which uses the system of the selective hybridization translation] After carrying out a blot to a nitrocellulose filter etc. and carrying out hybridization of the mRNA from the polypeptide production cell of lectin joint glycoprotein containing this invention glycoprotein 39, mRNA(s) corresponding to cDNA are collected. Collected mRNA Pouring to the oocyte of a protein translation system, for example, a platanna, It is made to translate into protein by cell free systems, such as rabbit reticulocyte lysate and a wheat germ, it detects using the antibody to the core protein of lectin joint glycoprotein containing this invention glycoprotein 39, and the target stock is obtained.
[0025] In addition, the antibody to the core protein of lectin joint glycoprotein containing this invention glycoprotein 39 used in the above-mentioned method can be created by a well-known method.
[0026] That is, solubilize the cell membrane of the tissue cell which has discovered this invention glycoprotein 39 first using a surface active agent, this is made to stick to the lectin joint agarose column which glycoprotein 39 can combine, and lectin joint glycoprotein is prepared.
[0027] As a cell membrane solubilization fraction separation means of various tissue cells For example, human cancer tissue and a human cell are given to the high-speed centrifugal separation of 100,000xg after crushing in a suitable buffer solution, the residue is dissolved in a Triton series surfactant, this is again given to the high-speed centrifugal separation of 100,000xg, and the method of extracting the supernatant liquid is mentioned.
[0028] As lectin used in order to separate lectin joint glycoprotein containing this invention glycoprotein

39 from the obtained cell membrane solubilization fraction for example, -- peanut beans lectin (Peanut agglutinin, PNA) is mentioned, and this lectin may use what is marketed -- for example, peanut beans -- the very thing -- you may use what carried out extraction separation by the well-known means. Lectin joint agarose may use what is marketed, can be made to be able to carry out coupling to agarose gel by the usual means, and can also be obtained.
[0029] Hapten sugar, for example, a lactose solution etc., is used for elution of lectin joint glycoprotein. As for the concentration of the eluate used here, 0.05-0.2M are desirable.
[0030] Next, after processing lectin joint glycoprotein containing this invention glycoprotein 39 with trifluoro methansulfonic acid (TFMS) or hydrogen fluoride and removing a sugar chain, Antiserum is extracted, after carrying out immunity of this to mites, such as a rabbit, with Freund's complete adjuvant, setting a still more suitable interval and carrying out immunity with Freund's incomplete adjuvant several times. Next, after mixing with the cell component obtained by heat-treating Escherichia coli and carrying out centrifugal separation, and the antiserum obtained by the above-mentioned at 4 degrees C, the polyclonal antibody for which it will ask if centrifugal separation is carried out can be obtained.
[0031] According to a conventional method, subcloning of this invention gene clone obtained in the above can be carried out to various plasmids. For example, EcoRI [the cDNA fragment containing this invention gene cut and refined] It is EcoRI similarly. It cut. What is necessary is just to intercalate in the cleavage site of cloning vectors, such as pUC18 [Yanisch-Perron, C., et al., Gene, 83, and p.103-119 (1985)]. Thereby, a desired recombinant plasmid can be obtained. [moreover, the amplification and the individualization of a recombinant plasmid by the introduction and this to the host of the recombinant plasmid obtained] collecting various kinds of methods generally used, for example, the cell which is mainly in a logarithmic growth phase, -- CaCl2 treatment -- natural -- DNA It changes into the status of being easy to incorporate, and can carry out by the method into which look this like [method] and a vector is made to incorporate.
[0032] in addition, various kinds of operations adopted in the above -- for example, -- a part -- DNA Chemosynthesis, cutting of a DNA strand, deletion, addition or the enzyme treatment aiming at binding, and DNA isolation, refining, replication, selection, etc. -- it can also creep -- a conventional method can be followed. More specifically, it is Above DNA. Agarose gel electrophoresis etc. can perform isolation refining.
[0033] [moreover, the determination of the base sequence of this invention gene obtained above] It is DNA with a suitable restriction enzyme. After digesting, Dideoxy procedure [Sanger and et al., Proc. Natl.Acad.Sci. USA, 74, and p.5463(1977)] It can carry out by the ********- Gilbert method [A.M. Maxam and W.Gilbert, Methods in Enzymology, 65, and p.499 (1980)] etc. Furthermore, the decision of the above-mentioned base sequence can be easily made also by using a commercial sequence kit etc.
[0034] The part and the corresponding amino acid sequence of a base sequence of this invention glycoprotein 39 gene obtained in this way are shown in the arrangement number 1 and the arrangement number 2. The number of a base sets 5' end to 1, and is given in the direction of 3' end from 5' end. The number of amino acid residue is given in the direction of a C terminal from the amino terminal, and is setting to 1 the amino acid by which a code is carried out first. The arrangement number 1 is the translation field of the length of 180 bases most located in 5' end among the translation fields of glycoprotein 39 gene which has determined arrangement, and is equivalent to the protein portion of 60 amino acids. This arrangement is considered to be the repetition arrangement (tandem repeats) field which makes one unit 60 bases (20 amino acid residue) similar to a PEM gene, and it seems that there is

individual difference in the number of repetitions. Moreover, the translation field of glycoprotein 39 gene shown in the arrangement number 2 is the length of 981 bases, and is equivalent to the protein portion of 327 amino acids. Arrangement leads to 3' side (amino acid sequence the C terminal side) repeatedly further in a base sequence, and the arrangement number 2 connects the arrangement of the arrangement number 1 to this.
[0035] [according to use of the obtained this invention gene] [with well-known general gene modification technology] conventionally [Science, 224, and p.1431(1984); Biochem.Biophys.Res. Comm., 130, and p.692(1985); Proc.Natl.Acad.Sci., USA, 80, and p.5990(1983); Reference], such as the EP patent public presentation No. 187991 gazette, The core protein of glycoprotein 39 can be manufactured and acquired easily and in large quantities. Moreover, a specific antibody can be created to the core protein of glycoprotein 39 using the core protein of the glycoprotein 39 obtained by doing in this way. Although an antibody is manufactured according to the manufacturing method of the usual polyclonal antibody and a monoclonal antibody It is also possible to obtain an epitope specific antibody from the polyclonal antibody to the core protein complex of glycoprotein 39 according to the method [Sience, 228, p.740-742 (1985)] of Weinberger (Weinberger) and others. An antibody is used for refining of glycoprotein 39 and its core protein, measurement, discrimination, etc.
[0036] moreover, [the core protein of the glycoprotein 39 which are obtained by carrying out like the above] The polypeptide which methionine combined with the amino terminal of the amino acid sequence shown in an arrangement table, and the intermediate to which the portion or all of transit peptide for glycoprotein 39 joined together or suffered a loss to the amino terminal of the above-mentioned amino acid sequence are also included. Naturally, this variation is obtained, for example by the modification after translation, or is set to the gene engineering procedure. DNA which changed the gene obtained from nature by methods, such as for example, site SUPESHIFIKKU mu TAGENESHISU, or varied by chemosynthesis methods, such as a phosphite triester method, It can compound or a gene can be compounded combining both. The component which has variation can be obtained by using these genes, including this in the vector of a microorganism and making it produce from the microorganism by which the transformation was carried out. Moreover, these protein can perform the displacement of some the amino acids, and the alteration of arrangement by variation of nature or population, with the function maintained. Therefore, glycoprotein 39 gene of this invention also includes the gene which carries out the code of the protein which has the various above-mentioned variation. the end of a gene code -- TAG and TAA etc. -- a termination codon can be added. Arbitrary codons can be chosen to each amino acid, without not restricting a gene code to the codon illustrated by the above-mentioned arrangement numbers 1 and 2, but changing an amino acid sequence. For example, what is necessary is just to follow the conventional method in consideration of the codon usage of the host who uses for gene recombination etc. [Nucl.Acids.Res., 9, and p.43-74 (1981)].
[0037]
[Effect of the Invention] If this invention glycoprotein 39 gene is used, the core protein of glycoprotein 39 can be manufactured easily and in large quantities. The glycoprotein 39 of this invention A human cancer organization especially gastric cancer, colon cancer, a pancreatic cancer, While a manifestation is accepted in hepatic carcinoma, an esophagus cancer, lung cancer, etc., the application as a tumor marker, an immunopathy marker, or various inflammatory disease markers is expected from it being discovered to secretory normal tissues, such as the stomach, a large intestine, and lungs.
[0038]

[Working example] Next, a work example is given and this invention is explained still in detail.
[0039] Preparation of work-example 1 this invention glycoprotein 39: Gastric cancer cell KATO-III It is the bottom CaClof ice-cooling2, and MgCl2 addition PBS about 2g. [PBS (+)] Beating is carried out in inside and it is this Potter-Elvehjem It homogenized with the homogenizer of type.
[0040] On the pellet which carried out high-speed centrifugality (105,000xg) of this crushing liquid at 4 degrees C for 1 hour, and removed that supernatant liquid, the 2% triton X-100, 0.15M NaCl and 0.01M tris HCl (pH 7.6) And 60ml of solutions containing PMSF (phenylmethyl sulfonyl fluoride) (sigma company) which is a 50microg/ml protease inhibitor are added. After homogenizing this furthermore, it was neglected at 4 degrees C for 30 minutes, and the cell membrane was solubilized. High-speed centrifugality (105,000xg) of this was carried out at 4 degrees C for 1 hour, and this supernatant liquid was obtained.
[0041] PNA of marketing of this supernatant liquid It adds in a joint agarose column (E. Y product made by lab RATORIZU), and is PNA. Joint glycoprotein was made to adsorb.
[0042] 200ml of penetrant removers (pH 7.6) containing the 0.1 % triton X-100, 0.15M NaCl, and 0.01M tris HCl washed this column. After that PNA It is 0.05M about joint glycoprotein. You made it eluted with 50ml of lactose solutions.
[0043] PNA in an eluate It measures with a Lowry method and the protein concentration of joint glycoprotein is 3mg (protein content) PNA in a total amount. Joint glycoprotein was obtained.
[0044] Work example 2 (1) PNA Sugar-chain removal of joint glycoprotein: PNA After freeze-drying 2mg of joint glycoprotein, 1ml of trifluoro methansulfonic acid (TFMS)-anisole (2:1) solutions were added, and it dissolved. After carrying out aeration of the nitrogen gas and replacing it in reaction mixture, it agitated at 25 degrees C for 5 hours, and the sugar chain was disassembled. After the end of a reaction, after adding diethylether of the amount of duplexes and mixing with it, it was neglected at -80 degree C for 1 hour. Next, an equivalent amount of ice-cooled 50% pyridine solutions were added, it agitated with the vortex mixer, and, subsequently the ether layer was removed. After adding ether furthermore and performing ether extraction twice similarly, it dialyzed to 2mM pyridine acetic acid buffer (pH 5.5) 4l.
[0045] (2) PNA Sugar chain removal PNA prepared by creation: (1) of the polyclonal antibody to the core protein of joint glycoprotein 0.5ml of PBS (-) solutions (protein concentration 800microg/ml) of joint glycoprotein 0.5ml of Freund's Freund's complete adjuvant Subcutaneous vaccination of the suspension mixed and prepared was carried out to **** of the buck hare of a New Zealand white kind. Freund's incomplete adjuvant and PNA of every two-week 3 times of after that, and above-mentioned Freund Subcutaneous vaccination of the suspension of joint glycoprotein was carried out to **** or the back, and immunity was carried out to it. After collecting blood from the ear vein of the rabbit and carrying out clot of blood completely after the last immunity on the 10th, the high-speed centrifugality (150,000rpm) during 20 minutes was repeated twice at 4 degrees C, supernatant liquid was collected, and antiserum was obtained.
[0046] (3) Absorption treatment of an antibody : not to carry out the cross reaction of the antibody which uses the core protein of this invention glycoprotein 39 shown in the after-mentioned work example 3 (2) for screening for the recombinant phage clonal separation which carries out a code to an Escherichia coli cell component is desired. Then, it is Escherichia coli (E.coli Y1090) about the antibody beforehand used for screening. It was made to react with a cell component and the antibody which intersects this was removed.

[0047] E. coli Y1090 [a stock] LB culture medium [Molecular Cloning (A Laboratory Manual); T. Maniatis, E.F.Fritsch, J.Sambrook; Cold Spring Harbor Laboratory (1982), p.68] 500ml Overnight culture was carried out at 37 degrees C in inside, and cells were collected by 5000rpm and the centrifugality for 10 minutes. This was suspended in 20ml of distilled water, and it heat-treated for 5 to 10 minutes by 100 **. Furthermore, 10,000rpm Supernatant liquid was separated after carrying out the at-long-intervals heart for 10 minutes. Next, work example 2 (2) [the created antiserum / 100ml of solutions diluted with PBS (-) 50 times] 10,000rpm after adding 1ml of this supernatant liquid, mixing with it and neglecting it at 4 degrees C for 2 hours The at-long-intervals heart was carried out for 15 minutes, that supernatant liquid was separated and the antibody to the core protein of this invention glycoprotein 39 was obtained.

[0048] Work example 3 (1) CDNA-library creation of gastric-cancer cell-strain KATO-III: It is gastric cancer cell strain KATO-III RPMI-1640 It is 5% of CO2 at the culture medium which added fetal calf serum to the culture medium at 10% of a rate. Subculture was carried out at 37 degrees C under gas aeration. From obtained gastric cancer cell strain KATO-III 1g to the GUANIJIUMU isothiocyanate method [Molecular Cloning; (A Laboratory Manual) T.Maniatis and E.F.Fritsch, J. Sambrook; Cold Spring Harbor Laboratory (1982), p. All the RNA 3mg is extracted according to 196], an oligo (dT) cellulose column (Colaborative Research Inc., capacity of column of 1ml) is used for this, and it is (Pori A) +RNA200microg. It obtained. According to the protocol of the cDNA synthesis system of Amersham, double-strand cDNA was compounded below. That is, reverse transcriptase (Amersham) is made to act on applicable (Pori A) +RNA 5microg, and it is the first DNA. The chain was compounded. Next, Escherichia coli ribonuclease H (RNase H) and Escherichia coli DNA Polymerase I (both Amersham) is made to act. RNA It is the first DNA, digesting. A chain is used as a mold and it is the second DNA. A chain is compounded and it is T4DNA. The double strand cDNA (ds-cDNA) which has a flush end using the exonuclease activity of polymerase was compounded.

[0049] ds-cDNA obtained by the above Cloning was further carried out to expression vector lambdagt11 using cDNA and cloning system lambdagt11 of Amersham. Namely, ds-cDNA EcoRI Methylase (Amersham) is made to act. ds-cDNA Restriction enzyme EcoRI in an inside A recognition site is protected by a methyl group. Next, T4DNA Connected the synthetic EcoRI linker (Amersham) to both ends with ligase (Amersham), the restriction enzyme EcoRI (Amersham) was made to act on this finally, and both ends were used as the cohesive end.

[0050] This ds-cDNA It is T4DNA about lambdagt11 arm (Amersham). It is made to join together with ligase (Amersham), and is a recombinant DNA. It created. In-vitro packaging liquid (Amersham) was made to act on this, and the cDNA library was created.

[0051] (2) Incubate the lambdagt11 cDNA library and E.coli Y1090 which were obtained by separation: (1) of the recombinant phage clone which carries out the code of this invention glycoprotein 39 for 20 minutes at 37 degrees C. Y1090 which is a host bacterium about recombinant phage After making it adsorb, it mixed with the dissolved upper soft agar, and it wound on the agar plate and extended. The agar plate after the upper agar solidification was cultivated at 42 degrees C for 4 to 8 hours, and the plaque was made to form. Subsequently, 10mM isopropyl 1-****- beta-D - Galactoside (IPTG) It was made to be saturated, and the dried nitrocellulose filter was put on the agar plate surface, it incubated at 37 degrees C for 2 hours, and beta-galactosidase fused protein was made to discover.

[0052] The filter was stripped after cooling this at 4 degrees C after that for 1 hour or more. this filter -- a room temperature -- a 1-hour blocking solution (2% horse hemoglobin --) 0.1 After dipping in %

Tween20 and PBS (-), it was made to react with the 50microg/ml antibody to the core protein of this invention glycoprotein 39 which carried out absorption treatment in the work example 2 (3) in this blocking solution, and was made to incubate at a room temperature for 2 hours. This filter by PBS (-) which contains Tween20 0.1% After 5 times washing, This filter was made to react at a room temperature for 2 hours in a horseradish par oxidase (HRP) label anti-rabbit IgG antibody (made by Cappel) blocking solution (200 time diluent), and the above-mentioned penetrant remover washed 5 times after this end of a reaction. Subsequently, the clone which was made to color with a hydrogen peroxide content 4-chloro 1-naphthol solution, and has discovered the fused protein corresponding to the core protein of this invention glycoprotein 39 was chosen. Y1090 after separating the single plaque of the obtained clone Made it increase as a host, and it was made to suspend in SM buffer solution, and saved at 4 degrees C. This clone was named lambdaKP39.

[0053] (3) [this invention glycoprotein 39] Lysogenic-bacterium creation of recombinant phage which carries out a code : Huynh, T. V., Young, and R.A., Davis, R.W. : According to the method of DNA Coloning Vol.1 A Practical Approach (ed.), Glover, D.M., and IRL Press (1985) p.49 -78 description, [lambdaKP39] E. The lysogenic bacterium which coli BNN103 were made to lysogenize was created.

[0054] (4) It is the recombinant phage clone (lambdaKP39) which carries out the code of this invention glycoprotein 39 obtained by separation: (2) of the recombinant phage DNA which carries out the code of this invention glycoprotein 39 E.coli Y1090 After making it increase as a host, [Molecular Cloning (A) Laboratory Manual; T.Maniatis, E.F.Fritsch, J.Sambrook; A method given in ColdSpring Harbor Laboratory (1982) p.371-372] is followed. this invention recombinant phage DNA (lambdaKP39 DNA) was prepared.

[0055] (5) Creation of plasmid pKP39 transformant : lambdaKP39 DNA was digested with the restriction enzyme EcoRI (made by a Japanese gene company), and the DNA fragment of about 1900 base pairs was obtained.

[0056] After, digesting plasmid vector pBluescript II KS (made by a SUTORATA gene company) by EcoRI similarly on the other hand, The piece of bisection was combined by T4 DNA ligase (made by TAKARA SHUZO CO., LTD.), and recombinant plasmid pKP39 which carry out the code of the polypeptide chain of this invention glycoprotein 39 were obtained.

[0057] Recombinant plasmid pKP39 obtained Transduction was carried out to the competent cell of E. coli JM83.

[0058] (6) pKP39 obtained by creation: (5) of the restriction enzyme map [Molecular Cloning (A) Laboratory Manual; T. Maniatis, E.F.Fritsch, J.Sambrook; Cold Spring Harbor Laboratory p(1982).104-106] It processes according to the method of a description. pKP39 which carry out the code of this invention glycoprotein 39 according to the method of the pan above-mentioned literature p.374-p.381 The restriction enzyme map of the clone was created (drawing 1).

[0059] (7) pKP39 Base sequence determination of a clone : pKP39 The determination of the base sequence of a clone is the method of Sanger (Sanger) and others. [Sanger F., Nicklen S.& Coulson A.R., Proc.Natl.Acad.Sci.USA, and 74, p. It carried out according to 5463-5467(1977)].

[0060] The arrangement of glycoprotein 39 gene obtained from the above result consists of about 1900 bases on the whole including a translation field and the untranslation region by the side of 3'. Among these, it is the repetition arrangement field where about 600 bases make 60 bases one unit from 5' end. Including this field, a translation field is the length of about 1560 bases, and it became clear that the code of the protein portion of about 553 amino acids was carried out. however, the arrangement of a

repetition arrangement field -- 180 bases (the code of the 60 amino acid residue can be carried out) -- it has determined (arrangement number 1) -- other repetition arrangement is undecided. From repetition arrangement, the arrangement of 1320 bases of an underline was determined and was shown in the arrangement number 2.
[0061] Work example 4 (1) The GUANIJIUMU isothiocyanate method shown in preparation work-example 3-(1) of all the RNA and (Pori A) +RNA is followed, and they are all the RNA from gastric cancer cell strain KATO-III. It extracts. Moreover, a commercial oligo (dT) cellulose column (Colaborative Research Inc.) is used, and it is (Pori A) +RNA. It prepared (said MolecularCloning p.196-198 reference).
[0062] (2) Follow the method of said Molecular Cloning (p. 200-201) in all the RNA20microg or (Pori A) +RNA10microg prepared by Northern blotting (1). It is 90V at the 1% agarose gel which contains 10mM sodium phosphate solution after warming for 1 hour and making it denaturalize at 50 degrees C, the bottom of glyoxal existence, and. Electrophoresis was performed for 3 to 4 hours. Next, separated RNA was made to transfer over 15 hours to a nitrocellulose filter (SHURAIA and shell company) in 20xSSC. RNA The nitrocellulose filter after transcription was baked at 80 degrees C after desiccation with the room temperature for 2 hours, and it fixed, and after that, among 20mM tris hydrochloric acid buffer (pH 8.0), it heated for 5 minutes in 100 **, and glyoxal was removed. It is this filter Work-example 3-(7) After shaking at 42 degrees C in the described pre hybridization solution for 3 hours, It moved into the hybridization solution (a presentation is the same as a pre hybridization solution except a probe) containing an alpha-32 P-dCTP label probe, and shook at 42 degrees C for 20 hours. A probe is pKP39. What carried out the label of the fragment which cut the inside cDNA of a clone with the restriction enzyme EcoRI in alpha-32 P-dCTP using the multi-prime DNA labeling system (Amersham) was used by 0.5 to 1x107 cpm/ml concentration. after the end of high bleeder IZESHON, and a filter -- 2xSSC-0.1% SDS moving to a solution and washing every 3 times during 10 minutes at a room temperature -- further -- 0.1xSSC-0.1% SDS After washing every 3 times during 30 minutes at 60 degrees C in a solution, it dried at the room temperature. The filter was stuck on the filter paper, it put into the X-ray film cassette, and the X-ray film (Konica Corp. XAR-5) was exposed for one to three days at -70 degrees C in piles.
[0063] The result of obtained Northern blotting is shown in drawing 2 .
[0064] In addition, RNA It is 28S as a molecular weight marker. And 18S Ribosomal RNA It used. As a result, two mRNA(s), 4400 base length and 6800 base length, were detected. This is an alternative like known PEM mRNA. It is thought that it produced by splicing.
[0065]
[Layout Table]
arrangement number: -- length [of 1 arrangement]: -- mold [of 180 arrangement]: -- number [of nucleic acid chains]: -- double strand topology: -- kind [of normal chain-like arrangement]: -- cDNA to mRNA origin living thing name: -- kind [of Homo sapiens cell]: -- stomach signet-ring-cell-carcinoma cellular in: -- KATO-III -- direct origin library name: -- lambdagt11 KATO-III cDNA library clone name: -- sign:mat peptide existence position: showing the feature feature of lambdaKP39 arrangement -- sign:repeat region existence position: showing the method:S feature which determined 1..180 feature -- [1..180 feature] The sign:repeat unit existence position showing the determined method:S feature : Method:S arrangement GGC TCC ACC GCC CCC CCA GCC CAC GGT GTC ACC TCG GCC CCG GAG AGC which determined 1..60 features 48Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro

Glu Ser 1 5 10 15 AGG CCG GCC CCG GGC = TCC ACC GCG CCC GCA GCC CAC GGT GTC ACC TCG 96Arg Pro Ala Pro Gly Ser Thr Ala Pro Ala Ala His Gly Val Thr Ser 20 25 30 GCC CCG GAG AGC AGG CCG GCC CCG GGC TCC ACC GCG CCC GCA GCC CAC 144Ala Pro Glu Ser Arg Pro Ala Pro Gly Ser Thr Ala Pro Ala Ala His 35 40 45 GGT GTC ACC TCG GCC CCG GAC ACC AGG CCG GCC CCG 180Gly Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro 50 = 55 60 [0066] arrangement number: -- length [of 2 arrangement]: -- mold [of 1320 arrangement]: -- number [of nucleic acid chains]: -- double strand topology: -- kind [of normal chain-like arrangement]: -- cDNA to mRNA origin living thing name: -- kind [of Homo sapiens cell]: -- stomach signet-ring-cell-carcinoma cellular in: -- KATO-III -- direct origin library name: -- lambdagt11 KATO-III cDNA library clone name: -- sign:mat peptide existence position: showing the feature feature of lambdaKP39 arrangement -- sign:polyA signal existence position: showing the method:S feature which determined 1..981 feature -- [1267..1272 feature] The sign:polyA site existence position showing the determined method:S feature : Method:S arrangement GGC TCC ACC GCC CCC CCA GCC CAC GGT GTC ACC TCG GCC CCG GAC ACC which determined 1293..1320 features 48Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr 5 10 15 AGG CCC GCC TTG GG= C TCC ACC GCG CCT CCA GTC CAC AAT GTC ACC TCG 96Arg Pro Ala Leu Gly Ser Thr Ala Pro Pro Val His Asn Val Thr Ser 20 25 30 GCC TCA GGC TCT GCA TCA GGC TCA GCT TCT ACT CTG GTG CAC AAC GGC 144Ala Ser Gly Ser Ala Ser Gly Ser Ala Ser Thr Leu Val His Asn Gly 35 40 45 ACC TCT GCC AGG GCT ACC ACA ACC CCA GCC AGC AAG AGC ACT CCA TTC 192Thr Ser Ala Arg Ala Thr Thr Pro Ala Ser Lys Ser Thr Pro Phe = 50 55 60 TCA ATT CCC AGC CAC CAC TCT GAT ACT CCT ACC ACC CTT GCC AGC CAT 240Ser Ile Pro Ser His His Ser Asp Thr Pro Thr Thr Leu Ala Ser His 65 70 75 80 AGC ACC AAG ACT GAT GCC AGT AGC ACT CAC CAT AGC ACG GTA CCT CCT 288Ser Thr Lys Thr Asp Ala Ser Ser Thr His His Ser Thr Val Pro Pr o 85 90 95 CTC ACC TCC TCC AAT CAC AGC ACT TCT CCC CAG TTG TCT ACT GGG GTC 336Leu Thr Ser Ser Asn His Ser Thr Ser Pro Gln Leu Ser Thr Gly Val 100 105 110 TCT TTC TTT TTC CTG TCT TTT CAC ATT TCA AAC CTC CAG TTT AAT TCC 384Ser Phe Phe Phe Leu Ser Phe His Ile Ser Asn Leu Gln Phe Asn Ser 115 120 125 TCT CTG GAA GAT CCC AGC ACC GAC TAC TAC CAA GAG CTG CAG A= GA GAC 432Ser Leu Glu Asp Pro Ser Thr Asp Tyr Tyr Gln Glu Leu Gln Arg Asp 130 135 140 ATT TCT GAA ATG TTT TTG CAG ATT TAT AAA CAA GGG GGT TTT CTG GGC 480Ile Ser Glu Met Phe Leu Gln Ile Tyr Lys Gln Gly Gly Phe Leu Gly 145 150 155 160 CTC TCC AAT ATT AAG TTC AGG CCA GGA TCT GTG GTG GTA CAA TTG ACT 528Leu Ser Asn Ile Lys Phe Arg Pro Gly Ser Val Val Val Gln Leu Thr 165 = 170 175 CTG GCC TTC CGA GAA GGT ACC ATC AAT GTC CAC GAC GTG GAG ACA CAG 576Leu Ala Phe Arg Glu Gly Thr Ile Asn Val His Asp Val Glu Thr Gln 180 185 190 TTC AAT CAG TAT AAA ACG GAA GCA GCC TCT CGA TAT AAC CTG ACG ATC 624Phe Asn Gln Tyr Lys Thr Glu Ala Ala Ser Arg Tyr Asn Leu Thr Ile 195 200 205 TCA GAC GTC AGC GTG AGT GAT GTG CCA TTT CCT TTC TCT GCC CAG TCT 672Ser Asp Val Ser Val S= er Asp Val Pro Phe Pro Phe Ser Ala Gln Ser 210 215 220 GGG GCT GGG GTG CCA GGC TGG GGC ATC GCG CTG CTG GTG CTG GTC TGT 720Gly Ala Gly Val Pro Gly Trp Gly Ile Ala Leu Leu Val Leu Val Cys 225 230 235 240 GTT GCG CTG GCC ATT GTC TAT CTC ATT GCC TTG GCT GTC TGT 768Val Leu Val Ala Leu Ala Ile Val Tyr Leu Ile Ala Leu Ala Val Cys 245 250 255 CAG TGC CGC = CGA AAG AAC TAC GGG CAG CTG GAC ATC TTT CCA GCC CGG 816Gln Cys Arg Arg Lys Asn Tyr Gly Gln Leu Asp Ile Phe Pro Ala Arg 260 265 270 GAT ACC TAC CAT CCT ATG AGC GAG TAC CCC ACC TAC CAC ACC CAT GGG 864Asp Thr Tyr His Pro Met Ser Glu

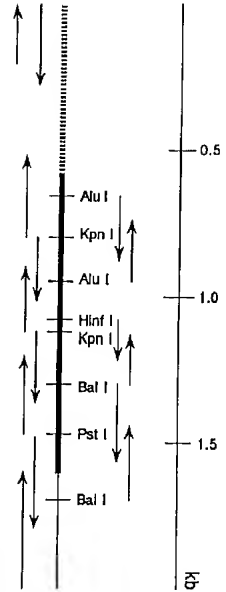
Tyr Pro Thr Tyr His Thr His Gly 275 280 285 CGC TAT GTG CCC CCT AGC AGT ACC GAT CGT AGC CCC TAT GAG AAG GTT 912Arg Tyr Val Pro Pro Ser Ser Thr Asp Arg Ser Pro Tyr Glu Lys Val 290 295 300 TCT GCA GGT AAT GGT GGC AGC AGC CTC TCT TAC ACA AAC CCA GCA GTG 960Ser Ala Gly Asn Gly Gly Ser Ser Leu Ser Tyr Thr Asn Pro Ala Val 305 310 315 320 GCA GCC ACT TCT GCC AAC TTG TAGGGGCACG TCGCCCGCTG AGCTGAGTGG 1011Ala Ala Thr Ser A= la Asn Leu 325 327 CCAGCCAGTG CCATTCCACT CCACTCAGGT TCTTCAGGGC CAGAGCCCCT GCACCCTGTT 1071 TGGGCTGGTG AGCTGGGAGT TCAGGTGGGC TGCTCACACC GTCCTTCAGA GGCCCCACCA 1131 ATTTCTCGGA CACTTCTCAG TGTGTGGAAG CTCATGTGGG CCCCTGAGGC TCATGCCTGG 1191 GAAGTGTTGT GGTGGGGGCT CCCAGGAGGA CTGGCCCAGA GAGCCCTGAG ATAGCGGGGA 1251 TCCTGAACTG GACTGAATAA AACGTGGTCT CCCACTGCGC CAAAAAAAAA AAAAAAAAAA 1311AAAAAAAAAA 1320

[Brief Description of the Drawings]

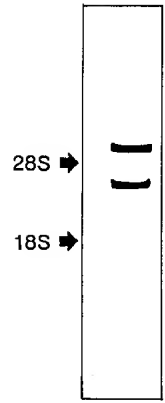
[Drawing 1] The restriction enzyme map and the base-sequence-determination method of cDNA which carry out the code of the core protein of this invention glycoprotein 39 are shown. The scale shown in the highest rung is the length (kilobase) of nucleotide on the basis of the 1st base of cDNA among drawing 1 . The lower berth is cDNA clonepKP39 which carries out the code of this invention glycoprotein 39. It is shown. The coding region which the thick linea-nigra portion of Chuo pulls to it the field which carries out the code of the repetition arrangement whose dashed line portion on the left-hand side of [this] a line top makes 20 amino acid residue one unit, and continues is shown. An arrow shows the direction and length of a base sequence which were determined about each DNA fragment.

[Drawing 2] They are the Drawings in which Northern blotting of mRNA which carries out the code of this invention protein in a work example 4 is shown. Two mRNA(s), 4400 base length and 6800 base length, exist.

[Drawing 1]



[Drawing 2]



[Translation done.]